

What is claimed is:

1. An electrochemical biosensor, comprising:

an insulating base plate;

5 a layer of electrically conductive wires disposed on said insulating base plate;

an electrode layer comprising electrodes having a reference electrode and at least one electrode, and two contact ports being adapted to connect a measuring device, and being formed on said layer of electrically conductive wires by screen printing except said reference electrode;

10 a middle insulating layer being applied on said conductive wire and said electrode layers without covering said electrodes and said contact ports, said insulating layer comprising an opening formed therein, and said opening being opposed to said electrodes of said insulating base plate;

15 an active reaction layer having substances of reactant, reaction catalyst, mediator and surfactant spread between the starting point of said opening and the surface of said electrodes; and

20 an upper cover opposing to an upwardly extended closed space formed within said insulating layer and being in contact above said insulating layer, said opening forming a capillary inflow area and said closed space being positioned opposing to one end of the inflow area.

2. The electrochemical biosensor according to claim 1, wherein said biosensor is a bi-electrode system comprising a working electrode and a reference electrode.

25 3. The electrochemical biosensor according to claim 1, wherein said biosensor is a tri-electrode system comprising a working electrode, a reference electrode and an auxiliary electrode.

4. The electrochemical biosensor according to claim 1, wherein said opening is U-shaped.
5. The electrochemical biosensor according to claim 1, wherein said opening is T-shaped.
- 5 6. The electrochemical biosensor according to claim 1, wherein the insulating base plate is made of material selected from the group consisting of polycarbonate, polyester, polyether, nylon, polyurethane, polyimide, polyvinylchloride (PVC), glass, glass fibre plate, ceramics and polyethylene terephthalate (PET).
- 10 7. The electrochemical biosensor according to claim 1, wherein said layer of electrically conductive wires is silver.
8. The electrochemical biosensor according to claim 1, wherein said layer of electrically conductive wires is silver chloride.
9. The electrochemical biosensor according to claim 1, wherein said layer of electrically conductive wires is gold.
- 15 10. The electrochemical biosensor according to claim 1, wherein said electrode layer is carbon.
11. The electrochemical biosensor according to claim 1, wherein said electrode layer is silver.
- 20 12. The electrochemical biosensor according to claim 1, wherein said electrode layer is gold.
13. The electrochemical biosensor according to claim 1, wherein said electrode layer is platinum.
14. The electrochemical biosensor according to claim 1, wherein said reaction catalyst is bio catalyst.
- 25 15. The electrochemical biosensor according to claim 1, wherein said reaction catalyst is non-bio catalyst.

16. The electrochemical biosensor according to claim 14, wherein said bio catalyst is enzyme.
17. The electrochemical biosensor according to claim 1, wherein the thickness of said middle insulating layer is between 20 and 400 μm .
- 5 18. The electrochemical biosensor according to claim 1, wherein the thickness of said middle insulating layer is between 50 and 200 μm .
19. The electrochemical biosensor according to claim 4, wherein the length and width of said opening is between 2 and 8 mm and between 0.5 and 5 mm, respectively.
- 10 20. The electrochemical biosensor according to claim 5, wherein the length and width of said opening is between 2 and 8 mm and between 0.5 and 5 mm, respectively.
- 15 21. The electrochemical biosensor according to claim 1, wherein the volume of said closed space opposing to said middle insulating layer is between 0.5 and 4 μl .
22. The electrochemical biosensor according to claim 1, wherein sample can be filled and detected when it is introduced above said working electrode and said auxiliary electrode.
- 20 23. The electrochemical biosensor according to claim 1, wherein said biosensor contains a device activation line which can activate the measuring device automatically.
24. The electrochemical biosensor according to claim 1, wherein said insulating material is applied on said conductive wire and said electrode layers by screen printing.
- 25 25. The electrochemical biosensor according to claim 1, wherein said insulating material is adhered on said conductive wire and said electrode layers.
26. The electrochemical biosensor according to claim 23, wherein said insulating material is insulating paste.

27. A method of electrochemically fabricating biosensor, comprising the steps of:

forming a layer of electrically conductive wires on a substrate by screen printing which then being dried between 40°C and 120°C;

5 forming an electrode layer on top of the layer of electrically conductive wires by screen printing and drying the substrate between 40°C and 120°C;

forming a middle insulating layer with a U-shaped opening formed therein above said electrode layer of the substrate by screen printing, wherein the working electrode, the reference electrode and the auxiliary electrode are confined within the U-shaped opening and the opposing ends of said electrodes are exposed to keep contact with the measuring device;

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applying an active reaction layer on said U-shaped opening;

adhering an upper cover with an opening formed therein above the middle insulating layer, wherein said opening is positioned at one end of said U-shaped opening; and

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applying a surface layer above said upper cover.

28. The method of claim 27, wherein the middle insulating layer can be formed on top of said electrode layer by adhesion instead of screen printing.

29. The method of claim 27, wherein said U-shaped opening can be replaced by a T-shaped opening and the transverse opening of said T-shaped opening forms two air vents on opposing sides of said biosensor.

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